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Marian Christopher

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the application of:

MILLER, et al

Serial No.: 08/487,312

Filing Date: June 7, 1995

For: BOVINE GROWTH HORMONE

Examiner: C. Saoud

Group Art Unit: 1646

Declaration of Raymond Bradley, CBE, MSc, BVetMed, FRCVS, FRCPath, CBiol, MIBiol
Pursuant to 37 C.F.R. § 1.132

MS Non-Fee Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

I, Raymond Bradley CBE, declare as follows:

Relevant Background Information

1. I am a veterinary surgeon and a trained veterinary pathologist. I was Head of Pathology Department at the Ministry of Agriculture, Fisheries and Food (MAFF, UK) Central Veterinary Laboratory (now known as the Veterinary Laboratories Agency – VLA) in 1986 when bovine spongiform encephalopathy (BSE) was discovered by two colleagues in the Department. I initiated and developed the initial BSE research programme with the aid of

colleagues in the following years and became increasingly involved in the national and international issues concerning the disease. I have been working in the field of BSE ever since.

2. I became BSE Co-ordinator for MAFF in 1991. I have advised the World Health Organisation (WHO), Office International des Epizooties (OIE), European Commission (EC), UK, Argentine, Japanese, Canadian and U.S. Governments via expert consultations, groups, committees, or on an individual basis on various aspects of transmissible spongiform encephalopathies (TSE). I retired as a full time employee of the VLA in 1995 and became a Private BSE Consultant. I continue to advise some of the above institutions and a number of trade associations and private companies on aspects of BSE and present scientific papers in international fora.

3. Based on my experience, I am qualified to analyse and reach an opinion concerning the TSE risk presented by methods of manufacturing bovine growth hormone (bGH).

4. In connection with preparing this declaration, I have reviewed the following materials: (1) U.S. Patent Application No. 487, 312 ("the '312 Application"); (2) the opinion of the U.S. Board of Patent Appeals and Interferences, Appeal No. 1998-1851 (March 26, 2003); (3) U.S. Patent No. 3,265,579 ("Daniels and Parcellas" (1996)); and (4) all of the materials cited in the references section at the conclusion of this declaration.

5. The following abbreviations are used in this declaration:

   a. **bGH** – bovine growth hormone also known as bovine somatotropin (bST).

   b. **BSE** – bovine spongiform encephalopathy.

   c. **CNS** - Central nervous system (brain, spinal cord and associated structures).

   d. **hGH** – human growth hormone.

   e. **MBM** - meat-and-bone-meal – an end product of rendering (cooking) unwanted or waste animal tissues. The other end product is tallow.

f. **Pituitary bGH or pit-bGH** – bGH derived from bovine pituitary glands.

g. **SAF** – scrapie-associated fibrils are morphologically distinct fibrils detectable by electron microscopy in detergent extracts of negatively-stained, TSE-infected brain material.

h. **Synthetic bGH** – bGH prepared by recombinant methods.

i. **TSE** – transmissible spongiform encephalopathy.

6. For the purposes of this declaration, the time period used to determine the TSE risk of synthetic and natural (pituitary derived) bGH is assumed to be from at least the start of the 1980s continuing until at least 1986 or 1987, following the first detection of BSE in cattle in the United Kingdom.

Bovine Growth Hormone (bGH)

7. bGH is a naturally occurring polypeptide hormone consisting of 191 amino acids produced by the anterior pituitary gland of domestic cattle *Bos taurus*. The gland synthesises and excretes the hormone throughout life, though more may be excreted during the juvenile growth phase. It has an anabolic natural function that controls growth processes notably of the musculo-skeletal system and also can improve lactation yields in dairy cows.

8. The pituitary gland consists of two parts, the anterior part and the posterior part. Growth hormone is generated in and secreted from the anterior portion of the pituitary gland. The pituitary gland is ventrally located and attached to the brain by a short stalk or infundibulum.

9. Pituitary bGH (bGH derived from pituitaries) has been used for research purposes. Production of natural bGH necessitates the use of pools of many bovine pituitaries to yield sufficient quantity of the hormone. The yield of bGH from pituitary glands of slaughtered cattle is insufficient to enable its use in large numbers of animals.

Transmissible Spongiform Encephalopathies (TSE)

10. TSE are infectious (*i.e.*, naturally or experimentally transmissible) diseases of man and animals that consistently are fatal (Brown *et al,* 2003). They are progressive nervous diseases, with incubation periods usually measured in

years or decades, and infected individuals show no conventional immune response.

11. The causal agent of TSE has not been definitively established. What is clear is that in all these diseases the normal form of a host membrane sialoglycoprotein called PrP^{C} or PrP-sen (because it is sensitive to protease digestion) is post-translationally misfolded into a partially protease resistant form known as prion protein, PrP^{Sc} or PrP-res. There are techniques available by which to examine animal tissues, particularly central nervous system (CNS) tissues, to determine the presence of the disease. For example, one can look for the characteristic lesions of spongiform encephalopathy by light microscopy, detect so-called scrapie associated fibrils (SAF) by electron microscopy or identify PrP-res by immunohistochemistry or immunoblotting. Although it is possible to test for infectivity by bioassay of tissue, this is currently not practical except in limited research situations as it usually takes over a year to obtain a result.

12. Bovine Spongiform Encephalopathy (BSE) is a naturally occurring form of TSE found in domestic cattle (Wells *et al*, 1987). BSE is a progressive neurological disorder, and is fatal. BSE is a new disease with the first clinical case occurring probably in 1985 (Wilesmith *et al,* 1988). Canada has reported three cases of BSE to date. One occurred in an animal imported from the U.K. and was confirmed in 1993. Two subsequent cases have occurred in native-born Canadian cattle in 2003. One of these had been exported to the U.S. and was confirmed as a BSE case in December 2003.

13. BSE appears to be caused by a single major strain of TSE agent (the BSE agent) with specific biological and molecular properties that have also been found naturally in isolates from brain tissue from humans with variant Creutzfeldt-Jakob disease (vCJD) (Bruce *et al,* 1997). vCJD in humans was first reported from the U.K. in 1996 (Will *et al,* 1996) and is presumed to have resulted from ingested bovine products infected, or contaminated with the BSE agent (Will, 2003).

14. Creutzfeldt-Jakob disease (CJD) is a human TSE (Brown *et al,* 2003). There are various forms of this human TSE including sporadic CJD (the cause of

which is not known), vCJD mentioned above, familial CJD that is determined by mutations in the *PRNP* gene, and iatrogenic CJD caused by medical accidents (*see below*).

Risk of BSE Associated with bGH Derived from Pituitaries

15. The natural form of bGH is derived from pools of pituitaries from slaughtered cows. The WHO lists the pituitary gland as a high infectivity tissue for the TSE agent (WHO 2003). Although experimental studies have not been done to detect the BSE agent in bovine pituitaries, the pituitary gland of sheep, goats and humans has been conclusively shown to be infected with a TSE agent where there is TSE infectivity in the brain itself.

16. To obtain the pituitary tissue used to prepare natural (pit) bGH, slaughtered cows would almost certainly have been stunned before killing, as in most western societies cattle are first stunned for welfare reasons before killing by bleeding out. The most common method of stunning is by use of a penetrating captive bolt pistol that penetrates the skull and damages the brain such that the animal is rendered temporarily unconscious. This process can cause significant damage to the brain, and emboli produced as a result can enter the circulation and thus contaminate parts of the body that otherwise might not be naturally infected with the BSE agent (if it was present in the brain) (Garland, Bauer and Bailey, 1996, Anil *et al,* 1999). Use of stun guns that inject air under pressure into the cranial cavity or the use of a long rod-shaped instrument to penetrate the stun hole (a process called pithing) may cause further damage to the brain and exacerbate these features.

17. Once the pituitaries are removed from the carcase the anterior part would likely be separated from the posterior part. Given the close proximity of the pituitary to the brain, it would be difficult to guarantee that the anterior pituitary gland was not contaminated with other parts of the brain. If BSE infectivity had been present in the brain, pituitary gland, or in material that might contaminate the anterior pituitary gland, the risk of contamination would be high. As noted above, cross contamination of the pituitary gland with brain or nervous tissue could be enhanced by penetrative stunning and pithing.

18. Following excision of sufficient pituitary tissue, an extraction process is initiated to maximize the recovery of bGH and reduce the presence of unwanted or undesirable components. Such a process might include the process described by Daniels and Parcellas (1966).

19. During the time period considered (from 1980 to after the mid 1980s) a range of physical methods were used in conjunction with chemicals to purify the crude extracts from pituitaries including filtration and chromatography. Although these techniques might remove at least a proportion of any TSE infectivity if present, they could not be guaranteed to remove all infectivity. This includes for the process described by Daniels and Parcellas (1966).

20. The risk of BSE contamination of natural bGH derived from pituitaries is enhanced by the fact that cattle can be pre-clinically (and perhaps sub-clinically) infected with the BSE agent. In experiments, cattle that have been subjected to high oral dose exposure to BSE-infected brain material begin to show clinical signs of disease at around 35 months post-challenge. Notably, however, in the natural disease the mean incubation period is 60 months (range 20 months to perhaps lifetime). Infectivity and the misfolded form of PrP can be detected in the brain some 3 - 6 months before clinical onset of disease (Wells, 2003). This means that a clinically healthy animal could be pre-clinically infected even in the brain (not to mention in other tissues) and could not be distinguished from an unexposed, uninfected individual (except by impractical and time-consuming testing).

21. Moreover, organs collected at death, such as the pituitary gland, could thus be infected even though the donor appeared clinically healthy. Bioassays of pituitary glands from goats and Suffolk sheep with natural scrapie have shown infectivity (Hadlow, *et al,* 1980, 1982) and over 150 people have been infected with CJD following medical treatment with natural human pituitary hormones (Preece, 1993; Frasier and Foley, 1994; Will, 2003).

22. In addition, subclinical infection has been described in experimental models of TSE in which high levels of infectivity and PrP-res exist in tissues including the CNS without clinical signs developing during the normal lifespan (Hill and Collinge, 2003a, b). Although not yet determined to exist in cattle with BSE,

the possibility cannot be ruled out and that possibility is another reason why use of pituitary glands from seemingly healthy cattle could give a false assurance that no TSE infectivity exists.

23. Given all of these factors - (a) the fact that natural bGH is derived from pituitary glands, (b) the fact that the pituitaries of sheep, goats and humans have been conclusively shown to be directly or indirectly (by cross contamination) infected with the TSE agent where there is TSE infectivity in the brain itself, (c) the fact that in cattle the brain can be detectably infected with TSE before clinical onset of the disease and (d) the fact that a subclinical state of disease can theoretically exist in cattle - the TSE risk associated with pituitary derived bGH seems clear. Indeed, even if all pituitary glands in a pool used to prepare natural bGH came from clinically healthy animals, or animals believed to be clinically healthy, it does not preclude an infected pituitary gland, or glands being present in the pool, either because they are infected, or because they have become accidentally cross-contaminated by infected material.

Use of Pituitary Derived bGH In Commercial Dairy Cattle Presents a Possible Vehicle for Transmission of BSE

24. As shown above, where bGH is derived from pituitaries there is a risk of BSE contamination in the hormone preparation.

25. Dairy cattle appear to be uniformly susceptible to BSE. As will be shown below, based on experimental evidence and historical fact, it is reasonable to conclude that the use of bGH in cattle in the U.S. would likely permit transmission of BSE if there were TSE infectivity in the administered hormone.

26. In current practice, a synthetic form of bGH is administered to dairy cattle in the U.S. to promote more efficient lactation. The bGH is inoculated sub-cutaneously at fortnightly intervals from not earlier than the 57th day of lactation until the end of lactation (Fetrow, 1995; IDFA, 2004; FDA 2004). Thus each recipient may receive around 15-20, 500mg doses during a lactation.

27. This route of inoculation has been effective in establishing TSE infection experimentally; it is more efficient than the oral route, although less efficient than the intracerebral or intravenous routes (Kimberlin and Walker, 1988, 1989).

28. It is known that inoculation of humans with human pituitary-derived hormones (including hGH and human gonadotrophin) has resulted in the transmission of TSE. For example, children of short stature were repeatedly inoculated subcutaneously with natural (pituitary-derived) hGH over a period of up to 14 years in the U.S., the U.K., France and some other countries and over 150 of those children developed CJD as a result of that treatment (Preece, 1993; Frasier and Foley, 1994; Brown *et al,* 2000; Will, 2003). Furthermore, Gibbs *et al* (1993) succeeded in experimentally transmitting CJD to a squirrel monkey from one vial of potentially contaminated lots of pituitary-derived hGH. For these reasons the natural form is no longer used. Instead recombinant (synthetic) hGH has been substituted. The date this was believed to have been done in the U.S. and U.K. was 1985. (Preece, 1993; Frasier and Foley; 1994; Brown *et al,* 2000).

29. Another example of iatrogenic transmission of TSE (scrapie) is in sheep. Scrapie is a natural TSE of sheep, goats, and moufflon. Iatrogenic-scrapie has occurred in the 1930s in sheep (Gordon, 1946), and again in the late 1990s in sheep and goats in Italy. (Capucchio *et al*, 1998 ; Agrimi *et al* 1999; Caramelli *et al*, 2001; Zanusso *et al*, 2003.) On each occasion a vaccine prepared using sheep tissues unknowingly contaminated with scrapie agent, probably from central nervous tissue, and administered by a parenteral route, was responsible.

30. Moreover, the likelihood that treatment of cows with pituitary-derived bGH would be a vehicle for TSE transmission is strengthened by the small amount of material needed to establish infectivity. In cattle, experiments have shown that about 1 mg of infected brain material can contain sufficient infectivity to induce disease by the oral route and that larger amounts of the same material (and thus larger doses from the same pool) shorten the incubation period. Parenteral routes including the subcutaneous route are more efficient than the

oral route (Kimberlin and Walker, 1988, 1989) and so a lower dose would likely be required to produce the same effect.

31. Based on the foregoing, it is more than likely that treatment of cattle with pituitary bGH would permit transmission of BSE if the starting material was infected or contaminated with a TSE agent.

Recombinant (Synthetic) bGH

32. It is my understanding that recombinant (synthetic) bGH does not contain and is not manufactured using any pituitary origin material. (*See* '312 Application.) This indicates a fundamental difference when compared with natural bGH. The significant risk of BSE is introduced into the natural, pituitary-derived hormone by the infected state of a cow donating a pituitary gland. With synthetic bGH, there is no BSE hazard, and therefore no BSE risk from pituitary material.

33. The recombinant methods used to produce synthetic bGH do involve the use of chemicals and reagents. It is my understanding that a second expert is opining regarding the relative safety of the recombinant processes used to prepare synthetic bGH. I assume for purposes of this analysis (but express no independent opinion regarding) his conclusion that the recombinant techniques present no risk of BSE infectivity.

Regulatory Significance

34. I have served as a consultant to regulatory agencies in Europe, Japan and the U.S. regarding BSE risk. I have advised these agencies, as well as a number of trade associations and private companies, on aspects of BSE including on safe sourcing of starting materials and processing methods for making vaccines prepared for use in humans.

35. Regulatory agencies are concerned about the risk of BSE infection and transmission in cattle. BSE transmission presents significant animal health, public health and economic risks to countries with BSE in their native-born stock.

36. In light of the risk of transmission arising from the derivation of bGH from pituitaries described above, it is my opinion that there is sufficient scientific justification for regulatory agencies to prohibit the use of the pituitary derived bGH in cattle. This conclusion is reinforced by the availability of a recombinant (synthetic) source of the hormone (synthetic bGH) for which there have been no reports of actual, or possible TSE transmission in any species and which in the light of scientific evidence is most unlikely to ever present a TSE risk.

Declaration

37. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both under Section 1001 of Title 18 of the United States Code, and such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

Date 23 March 2004

Signed: R C Bradley

Raymond Bradley CBE

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